Size Exclusion Chromatography of Polymers

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Introduction

In the characterization of polymers, size-exclusion chromatography (SEC) has become a standard technique for determining molar mass averages and molar mass distributions (MMD) of polymers. The principle of SEC is easily understood: owing to limited accessability of the pore volume within the particles of the column packing, polymer molecules are separated according to their hydrodynamic volumes, which can be correlated to their molar mass. Unfortunately, this is not as easy and simple as it looks. There are numerous sources of error and pitfalls in the separation, in the detection and in the treatment of the raw chromatographic data.

Separation

In SEC, the separation should be solely governed by size exclusion, which need not always be the case. Let us first consider the ideal case, in which size exclusion does govern the separation.

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Separation by Exclusion

Elution volumes in ideal SEC are limited to a narrow range (smaller than the column volume). It is clear, that no fraction of the sample can be eluted, before the interstitial volume V_i (i.e. the volume of the solvent outside the particles of the column packing) has passed the column.

- Large molecules, which have no access to the pores (exclusion limit), are eluted at V_i.
- Small molecules, which have access to the entire pore volume V_p, will appear at the void volume V_0, which is the sum of the interstitial volume V_i and the pore volume V_p.
- Molecules of a size between these extremes have access only to a part of the pore volume, hence they will be eluted with an elution volume V_e between V_i and V_0, which is determined by K_{SEC}, the equilibrium constant of a sample in ideal size exclusion chromatography:

$$V_e = V_i + K_{SEC} V_p$$

Obviously, elution volumes in ideal SEC are always smaller than the void volume of the column:

$$V_i < V_e < V_0 = V_i + V_p$$

$$0 < K_{SEC} < 1$$

The relation between V_e and the molar mass of a polymer is determined by calibration, as will be discussed later.

Exclusion Versus Nonexclusion Effects

The equilibrium constant of a chromatographic separation can be correlated with thermodynamic parameters.

The driving force for a separation at the (absolute) temperature T is the change in Gibbs Free Energy ΔG, which results from the changes in enthalpy (ΔH) and entropy (ΔS), respectively:

$$\Delta G = \Delta H - T\Delta S = -RT \ln K$$

In ideal SEC, which should be governed solely by entropy, ΔH should equal zero, and the equilibrium constant K_{SEC} should be given by:

$$K_{SEC} = e^{\Delta S/R}$$

while the equilibrium constant of adsorption chromatography (in the absence of exclusion) is determined by the enthalpy (typically, K_{LAC} is much larger than unity):

$$K_{LAC} = e^{-\Delta H/RT}$$

However, adsorption effects cannot always be completely eliminated. The distribution coefficient will then be given by:

$$K = K_{SEC} K_{LAC}$$

Under certain conditions, entropic and enthalpic phenomena will compensate each other, and K will equal 1. In this case, which is called the critical point of adsorption, all chains of the same structure are eluted in a narrow peak and separation of groups different from the repeating unit (end groups, branching sites, etc.) can be achieved.

This effect can be used in the analysis of functional oligomers or block copolymers. Even in the absence of adsorption phenomena there may be

another effect, which is called secondary exclusion. It originates from (electrostatic) repulsion of polar groups and has nothing to do with molar mass. The equilibrium constant K can then be divided into contributions from ideal size exclusion and from repulsion:

$$K = K_{SEC}K_{rep}$$

If interactions with the stationary phase also occur, the elution volumes will depend on all three distribution coefficients:

$$V_e = V_i + V_pK_{SEC}K_{rep}K_{LAC}$$

In ideal SEC, repulsion and adsorption should be absent, hence K_{rep} as well as K_{LAC} should equal unity.

The column(s)

Unlike other modes of high-performance liquid chromatography (HPLC), separation efficiency comes only from the stationary phase, while the mobile phase should have no effect. The whole separation occurs within the volume of the pores, which typically equals approximately 40% of the total column volume. This means, that long columns, or often sets of several columns are required. Different types of SEC columns are available, which are either based on porous silica or on semi-rigid (highly cross-linked) organic gels, in most cases copolymers of styrene (St) and divinylbenzene (DVB).

Table 1 lists some commercially available column packings for nonaqueous SEC. In general, silica-based packings are quite rugged, while organic packings must be handled with care. Typically, the separation range of a column covers approximately two decades of molar mass (e.g. from 1×10^4 to 1×10^6). Most producers also offer mixed bed columns covering a much wider range.

Obviously, a good separation is only one part of a good analysis. Another crucial point is the detection of the fractionated sample leaving the column.

Detection

Among the numerous HPLC detectors, only a limited number can reasonably be applied in SEC. One has to distinguish between concentration detectors and molar mass detectors.

- The response of a *concentration detector* is determined by the concentration of the solute in the mobile phase.
- The response of a *molar mass detector* depends on molar mass of the solute, as well as on concentration.

At least one concentration-sensitive detector has to be used in an SEC system. In the analysis of copolymers, a second concentration-sensitive detector is required, the sensitivity of which towards the components of the polymer differs from that of the first detector.

Concentration-sensitive Detectors

The concentration detectors most frequently used in SEC of polymers are the UV and the RI detectors. Infrared (IR) detection suffers from problems with the absorption of the mobile phase. Two other detectors are useful in the analysis of non-UV absorbing polymers: the density detector and the evaporative light-scattering detector (ELSD).

The UV detector detects UV-absorbing groups in the polymer, which may be the repeating unit, the end groups, or both. Hence, one has to distinguish two situations. For polymers, in which the repeating units contain a chromophoric group, the response of the UV detector represents the mass eluted in a given volume interval; for polymers with chromophoric end groups, however, the response depends on the number of polymer molecules. Many chromatographers use this assumption, when they derivatize 'nonabsorbing' polymers with UV-active reagents. Detection wavelengths are in the range of 180–350 nm but many SEC solvents allow detection only above a wavelength of 250 nm. Diode array detectors allow the measurement of an entire UV-spectrum at any point of the chromatogram, which is useful in SEC of copolymers.

Table 1 Column packings for nonaqueous size exclusion chromatography

Producer	*Packing*	*Material*
Rockland Technologies	Zorbax PSM	Porous silica microspheres
Shodex	Asahipak GF HQ	Highly crosslinked polyvinyl alcohol
Macherey-Nagel	Nucleogel GPC	St-DVB copolymer
Merck	LiChrogel PS	St-DVB copolymer
Phenomenex	Phenogel	St-DVB copolymer
Polymer Laboratories	PL gel	St-DVB copolymer
Waters	Styragel HR, HT, MW, Ultrastyragel	St-DVB copolymer
Jordi	Jordi GPC	100% DVB polymer

The ELSD detects any nonvolatile components of a sample. In this instrument, the eluate is nebulized and the solvent evaporated from the aerosol. Each droplet containing nonvolatile material will form a particle, which scatters the light of a transversal light beam. The response of this instrument for copolymers is, however, unclear – it depends on various parameters and the nature of these dependencies is rather complex. Moreover, lower oligomers may be strongly underestimated.

The refractive index (RI) detector exists in various modifications. It is the most common instrument in SEC besides the UV detector.

The density detector, which has been developed in the author's group, uses the principle of the mechanical oscillator. The measuring cell of this instrument is an oscillating, *U*-shaped capillary, the period of which depends on its reduced mass, and thus on the density of its contents. The signal of the density detector is thus inherently digital, and its response is integrated over each measuring interval.

Molar Mass-sensitive Detectors

The response of such a detector depends on the concentration and also on the molar mass of the fraction, hence it has to be combined with a concentration-sensitive detector. The following types of molar mass-sensitive detectors are on the market: low angle light scattering detectors (LALLS); multi-angle light scattering detectors (MALLS); and differential viscosimeters.

From light-scattering detection, the absolute molar mass distribution (MMD) can be determined directly but no information is obtained on polymer conformation. However, SEC with viscosity detection yields the intrinsic viscosity distribution (IVD). Hence it makes sense to combine a light-scattering detector with a viscosimeter detector. In light-scattering detectors (LALLS, MALLS) the light of a laser beam is scattered by the dissolved polymer coils in the measuring cell and the intensity of the scattered light is measured at angles different from zero.

The (excess) intensity $R(\theta)$ of the scattered light at the angle θ is correlated to the weight average of molar mass M_w:

$$\frac{K^*c}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2c$$

where c is the concentration of the polymer, A_2 is the second virial coefficient, and $P(\theta)$ describes the scattered light's angular dependence. K^* is an optical constant containing Avogadro's number N_A, the wavelength λ_0 refractive index n, and the refractive index increment dn/dc:

$$K^* = 4\pi^2(dn/dc)^2/(\lambda^4 N_A)$$

In a plot of $K^*c/R(\theta)$ versus $\sin^2(\theta/2)$, M_w can be obtained from the intercept and the radius of gyration from the slope. It must be mentioned, that dn/dc of copolymers may vary strongly with composition.

Viscosity detectors yield the intrinsic viscosity $[\eta]$, the so-called limiting viscosity number, which is defined as the limiting value of the ratio of specific viscosity ($\eta_{sp} = (\eta - \eta_0)/\eta_0$) and infinitely low concentration c:

$$[\eta] = \lim_{c \to 0} \frac{\eta - \eta_0}{\eta_0} = \lim_{c \to 0} \frac{\eta_{sp}}{c}$$

Since the concentrations in SEC are typically very low, $[\eta]$ can be approximated by η_{sp}/c. In viscosity detection, one has to determine the viscosity, η, of the sample solution as well as the viscosity, η_0, of the pure mobile phase.

This is typically done by measuring the pressure drop across a capillary; the pressure drop is proportional to the viscosity of the streaming liquid (using a differential pressure transducer). The problems of single capillary viscometers (SCV) are obvious: the viscosity changes, $\Delta\eta = \eta - \eta_0$, will typically be very small compared to η_0. Moreover, pressure differences due to pulsations of a reciprocating pump will be much larger than those resulting from the viscosity change caused by the eluted polymer.

A better, but still not perfect approach is the use of two capillaries in series, each of which is connected to a differential pressure transducer and a sufficiently large hold-up reservoir in between. Pulsations will be eliminated in this setup, because they appear in both transducers simultaneously.

A sophisticated approach is used in a differential viscometer, which is commercially available from Viscotek. In this instrument, four capillaries are arranged similar to a Wheatstone bridge. The detector measures the pressure difference ΔP at the differential pressure transducer between the inlets of the sample capillary and the reference capillary, which have a common outlet, and the overall pressure P at the inlet of the bridge. The specific viscosity, $\eta_{sp} = \Delta\eta/\eta$ is thus obtained from $\Delta P/P$.

Data Processing

Provided that the separation itself is reliable (which cannot always be taken for granted), the subsequent transformations are subject to errors:

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- Elution time to elution volume: because of the logarithmic relation between molar mass and elution volume, small changes of the flow rate can cause large errors in molar mass.
- Elution volume to molar mass: the molar mass of a fraction can be obtained either from calibration or from a molar mass-sensitive detector (in addition to the concentration detector).
- Detector response to polymer concentration: this requires a sufficiently wide linear range, a well-defined response of the detector(s) along the entire peak (i.e. for all molar masses within the MMD) and, in the case of copolymers, a second concentration detector.

Determination of Elution Volumes

The absolute flow rate can be obtained by measuring the time to fill a calibrated flask or by weighing the solvent passing the system in a defined time.

The knowledge of the absolute flow rate is, however, not completely necessary as long as flow rate variations are compensated by using an internal standard. The corrected flow rate is obtained from the ratio of the elution times of this standard peak. Such a correction only works well, if the flow rate is sufficiently constant within the entire chromatogram.

Determination of Molar Mass

Unless a molar mass-sensitive detector is used, one has to determine the molar mass of a fraction eluting at the volume V_e from a calibration, which can be obtained in different ways.

Calibration with narrow standards If a series of standards with a narrow MMD is available, their elution volumes must be determined to establish a calibration from which the molar mass for a given elution volume may be obtained. In the early years of SEC, a linear relation between $\log M$ and V_e was assumed, which is, however, only a first approximation, the quality of which depends very strongly on the columns:

$$\log M = A + BV_e$$

where A and B are constants, which can be determined very easily by linear regression. For many columns, the calibration line is, however, sigmoidal rather than linear (**Figure 1**). In this case, a polynomial fit matches the experimental points much better:

$$\log M = A + BV_e + CV_e^2 + DV_e^3 + EV_e^4 + \cdots$$

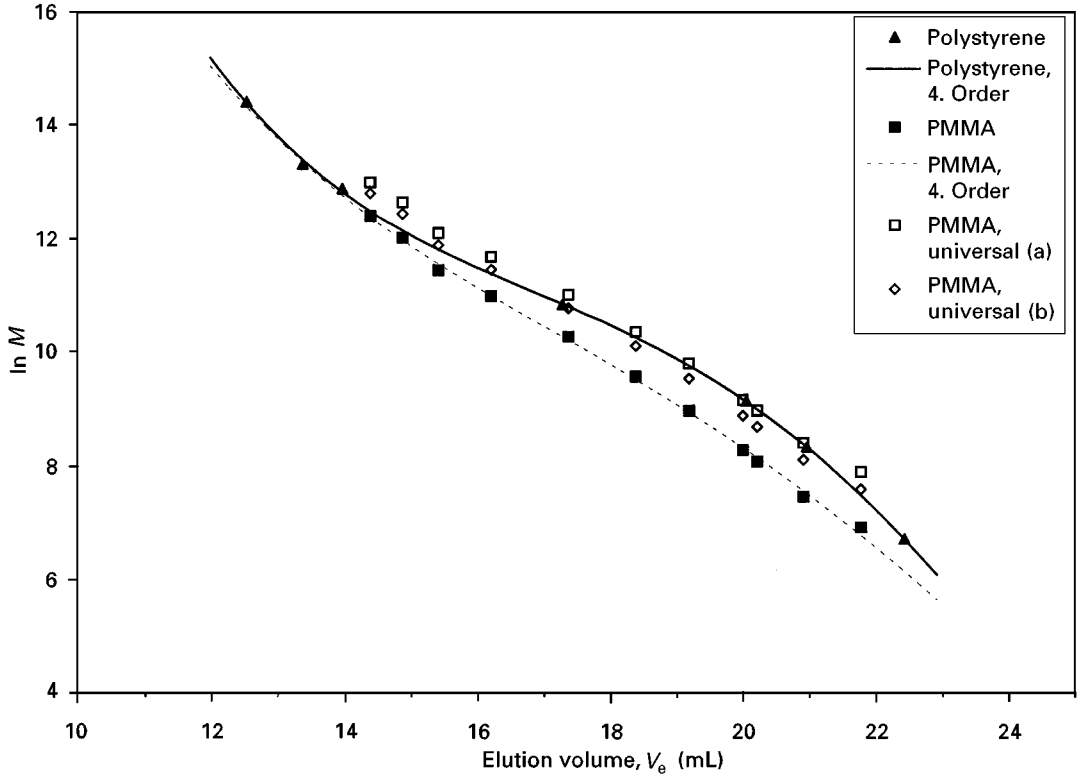

Figure 1 SEC calibration of a mixed bed column (Phenogel M, 7.8 × 600 mm) in 2-butanone at 25°C.

The order of the polynomial fit is, however, critical in some cases: if the number of data points (i.e. the number of standards) is too small, a fit of a too high order may produce an erroneous calibration line.

It must also be mentioned that there can be considerable differences between the calibration lines for different polymers on the same column in the same mobile phase.

This is especially important in the analysis of copolymers or polymer blends. Consequently, different molar masses will elute at the same volume, when a mixture of two homopolymers is analysed by SEC. The elution volume of a copolymer should be between the elution volumes of the homopolymers of the same molar mass.

If the composition of the copolymer at each point of the peak is known, a good approximation will be achieved by interpolation between the calibration lines. Calibration with narrow standards can be applied to many types of polymers, because appropriate standards have become commercially available for many polymers. In the low molecular range, additional data points can be taken from the maxima of oligomer peaks, which are at least partly resolved.

Different approaches have been described in the literature for the analysis of samples, for which no narrow MMD standards are available.

Calibration with broad standards If a well-characterized sample with broad MMD is available, different procedures may be used to establish a calibration fitting these averages.

The integral-MMD method can be applied if the entire MMD of the standard is known with high accuracy (which is, however, seldom the case). The method assumes, that the MMD of the sample can be described by the most probable distribution function and matches the calibration to this distribution. No assumptions on the shape of the calibration are made and the precision of the method is rather poor.

If only the molar mass averages of the sample are known from independent methods (light scattering or osmometry), linear calibration methods can be applied. The assumption of a linear calibration may, however, lead to erroneous results.

Universal calibration A very elegant approach is based on the fact, that in SEC the elution volume V_e of a polymer depends on its hydrodynamic volume, which is proportional to the product of its molar mass M and intrinsic viscosity $[\eta]$.

In a plot of log $(M[\eta])$ versus V_e (obtained on the same column), identical calibration lines should be found for two polymers (1 and 2), which can be considered as universal calibration:

$$M_1[\eta]_1 = M_2[\eta_2]$$

The intrinsic viscosity is a function of molar mass, which is described by the Mark–Houwink relationship, where K and a are constants for a given polymer in a given solvent at a given temperature:

$$[\eta] = KM^a$$

Combination of the equations above yields:

$$K_1 M_1^{a_1+1} = K_2 M_2^{a_2+1}$$

If a column has been calibrated with one polymer (1), the calibration line for another polymer (2) can be calculated, provided that the constants K and a are known for both polymers with sufficient accuracy:

$$\ln M_2 = \frac{1}{1+a_2} \ln \frac{K_1}{K_2} + \frac{1+a_1}{1+a_2} \ln M_1$$

The concept of universal calibration will also provide an appropriate calibration for polymers, for which no narrow standards exist.

The problem is, however, that the accuracy of K and a is limited even with polymers for which a sufficient number of well-defined standards exists: there are very large variations in the values reported in literature even in this case, as can be seen from **Table 2**.

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Table 2 Mark–Houwink–Sakurada parameters from literature[a] for common polymers in different solvents at 25°C

		K	a
$CHCl_3$, 25°C			
Polyethyleneglycol		0.20600	0.50
Poly(methyl methacrylate)	a	0.00581	0.79
	b	0.00480	0.80
	c	0.00340	0.83
Polystyrene	d	0.00716	0.76
	e	0.01120	0.73
2-Butanone, 25°C			
Poly(methyl methacrylate)	a	0.00680	0.720
	b	0.00710	0.720
	c	0.00680	0.830
	d	0.00939	0.680
Polystyrene	e	0.03900	0.580
	f	0.03050	0.600
	g	0.01950	0.635

[a]Kurata M, Tsunashima Y, Iwama M and Kamada K (1975) In: Brandrup J and Immergut E (eds), *Polymer Handbook*. New York: Wiley, p. IV–1.

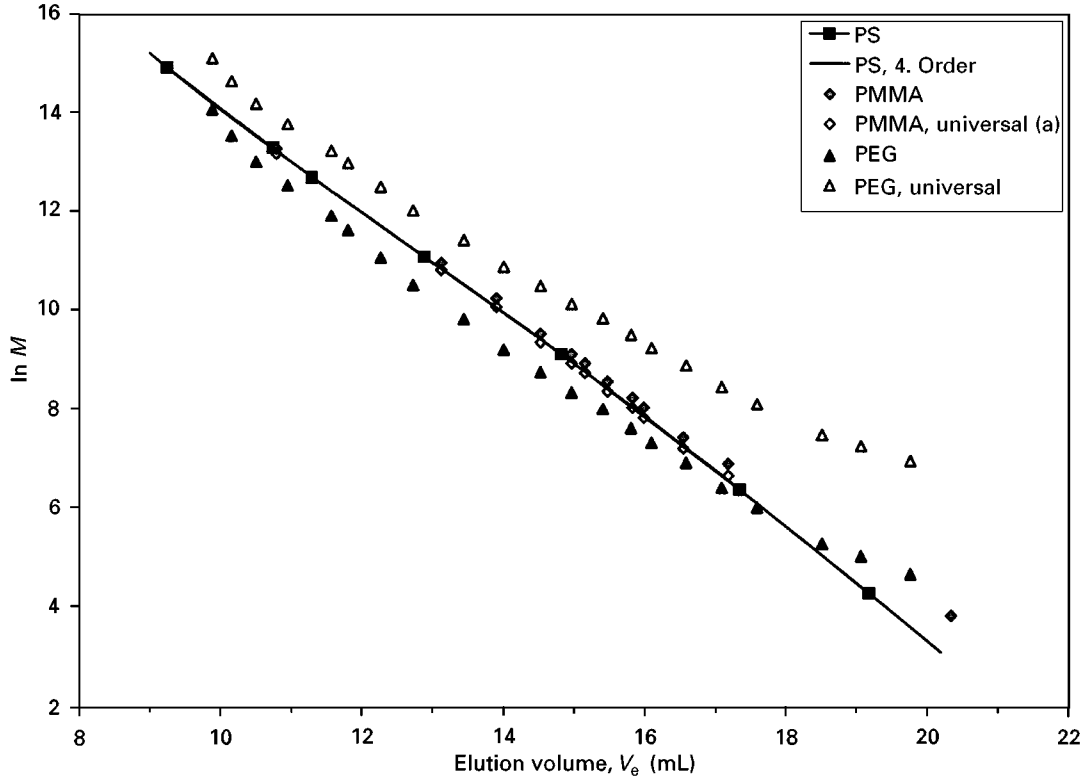

Figure 2 SEC calibration of a mixed bed column (PL Microgel M, 7.8 × 600 mm) in chloroform at 25°C.

The consequences can be dramatic, as is shown in **Figures 1** and **2**. In 2-butanone, the calibration functions obtained on a Phenogel M™ (Phenomenex) (mixed bed) column are quite different for polystyrene (PS) and poly(methyl methacrylate) (PMMA) (Figure 1). While the PS calibration is rather sigmoidal, a nearly linear calibration is found for PMMA. Using the MHS parameters from the literature (Table 2), both calibrations are closer, but there are still considerable differences.

In Figure 2, the calibrations of a PL Microgel M™ (Polymer Laboratories) (mixed bed) column are shown, which were obtained with PS, PMMA and PEG standards in chloroform. For PS and PMMA, very nice linear calibration functions are found, which almost coincide, while the attempt to establish a universal calibration for PEG with literature data makes the deviations even larger.

Quantification in SEC

After the first two transformations – time to volume and volume to molar mass – have been performed, there remains the third transformation – detector response to amount of polymer in a fraction – which can also be subject to errors, depending on the nature of the samples. In the following section, the particular problems are referred to with respect to the type of polymer to be analysed.

Molar Mass Dependence of Response Factors

In chromatography of polymers, the most frequently used detectors are the UV and the RI detector. Recently, we have introduced the density detector, which is very useful in the analysis of non-UV absorbing polymers.

The UV-detector responds to UV-absorbing groups in the polymer, which may be the repeating unit, the end groups, or both.

RI and density detectors measure a property of the entire eluate, that means, they are sensitive to a specific property of the sample (the RI increment or the apparent specific volume, respectively). It is a well known fact, that specific properties are related to molar mass:

$$x_i = x_\infty + \frac{K}{M_i}$$

where x_i is the property of a polymer with the molecular weight M_i, x_∞ is the property of a polymer with infinite (or at least very high) molecular weight, and

K is a constant reflecting the influence of the end groups.

A similar relation holds for the response factors for RI and density detection:

$$f_i = f_\infty + \frac{K}{M_i}$$

In a plot of the response factor, f_i, versus the molecular weight, M_i of a polymer homologous series (with the same end groups) one will obtain a straight line with the intercept f_∞ (the response factor of a polymer with very high molecular weight, or the response factor of the repeating unit) and the slope K, which represents the influence of the end groups.

Once f_∞ and K have been determined, the response factors for each fraction eluting from the column can be calculated using this equation (with the molecular weight obtained from the SEC calibration).

One more problem concerns preferential solvation: when a polymer is dissolved in a mixed solvent, the composition of the latter within the coils can be different from outside because of different interactions of the polymer chain with the individual components of the solvent. When the sample is separated on the column from the zone where the solvent would elute, a system peak (vacancy peak) appears, which is due to the missing component of the mobile phase. Obviously, the missing amount of solvent in the system peak appears in the peak of the polymer, the area of which is now different from what it would be in absence of preferential solvation.

Although this effect has been known for a long time, it is often neglected by chromatographers because they consider their mobile phase to be a 'pure' solvent, which is, however, generally not the case: even HPLC-grade solvents are seldom more than 99.5% pure and, if so, they take up moisture from the air, form peroxides and so on. The content of a second component (e.g. water or a stabilizer) can thus be much higher than the concentration of the sample when it leaves the column and enters the detector.

If the polarity of the end groups of a polymer is considerably different from that of the repeating units, their contribution cannot be neglected, and preferential solvation depends on molar mass.

Copolymers and polymer blends In the analysis of copolymers, the use of multiple detectors is generally inevitable. If the response factors of the detectors for the components of the polymer are sufficiently different, the chemical composition along the MMD can be determined from the detector signals.

When multiple detection is used, one has to be aware of errors arising from peak spreading between the detectors and from inaccurate shift time (just as in combinations with molar mass detectors). Typically, a combination of UV and RI detection is used, but other detector combinations have also been described.

If the components of the copolymer have different UV-spectra, a diode array detector will be the instrument of choice. However, one has to keep in mind, that nonlinear detector responses may also occur with UV detection. In the case of non-UV absorbing polymers, a combination of RI and density detection yields the desired information on chemical composition. The ELSD is not equally suitable because of unclear response to copolymers.

The principle of dual detection is quite simple: when a mass m_i of a copolymer, which contains the weight fractions w_A and w_B ($w_B = 1 - w_A$) of the monomers A and B, is eluted in the slice i of the peak, it will cause a signal $x_{i,j}$ in the detectors, the magnitude of which depends on the corresponding response factors $f_{j,A}$ and $f_{j,B}$, where j denotes the individual detectors:

$$x_{i,j} = m_i(w_A f_{A,j} + w_B f_{B,j})$$

The weight fractions w_A and w_B of the monomers can be calculated using:

$$\frac{1}{w_A} = 1 - \frac{\left(\frac{x_1}{x_2} * f_{2,A} - f_{1,A}\right)}{\left(\frac{x_1}{x_2} * f_{2,B} - f_{1,B}\right)}$$

Once the weight fractions of the monomers are known, the correct mass of polymer in the slice can be calculated using:

$$m_i = \frac{x_i}{w_A*(f_{1,A} - f_{1,B}) + f_{1,B}}$$

and the molecular weight, M_C of the copolymer is obtained by interpolation between the calibration lines of the homopolymers:

$$M_C = M_B + w_A*(M_A - M_B)$$

wherein M_A and M_B are the molecular weights of the homopolymers, which would elute in this slice.

A typical example is given in **Figure 3**, which shows the MMD of a block copolymer of ethylene oxide (EO) and propylene oxide (PO), as obtained by SEC (in chloroform) with coupled density and

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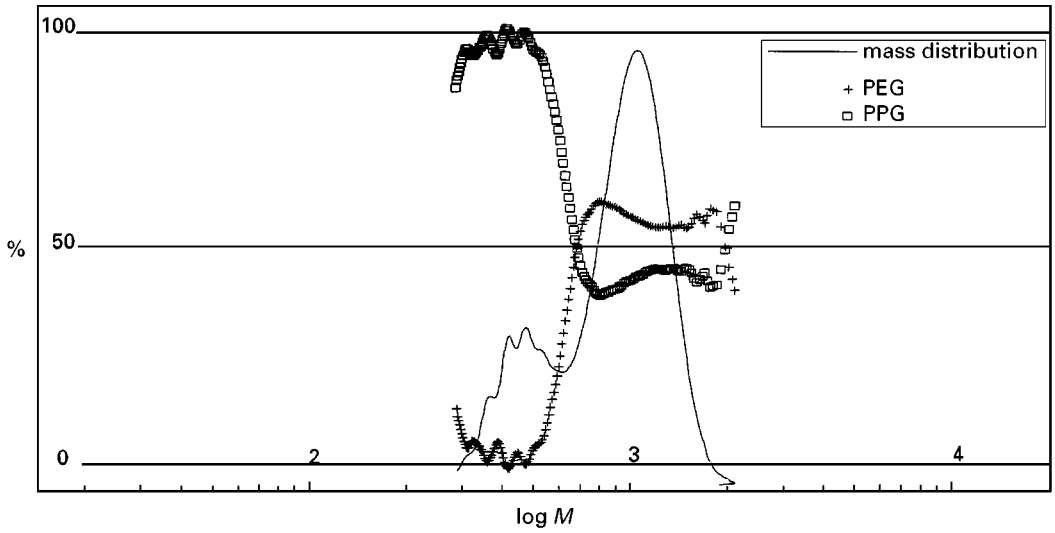

Figure 3 MMD and chemical composition of an EO–PO block copolymer (Figure 2), as determind by SEC in chloroform with density and RI detection. PEG + PPG.

RI detection. As can be clearly seen, this sample obviously contains polypropylene glycol as a by-product.

The interpolation between the calibration lines cannot be applied to mixtures of polymers (polymer blends): if the calibration lines of the homopolymers are different, different molecular weights of the homopolymers will elute at the same volume.

The universal calibration is not capable of eliminating the errors originating from the simultaneous elution of polymer fractions with the same hydrodynamic volume, but different composition and molar mass. Obviously it is feasible to use a combination of molar mass-sensitive detectors, such as a LALLS, MALLS and viscosity detector with two concentration detectors, from which the (average) composition for each fraction can be obtained, and thus the amount of polymer in the fraction. Nevertheless, discrimination of copolymers and polymer blends is impossible with one-dimensional chromatography.

Conclusion

The potential of SEC in polymer characterization is very great, especially when this technique is combined with other modes of liquid chromatography. Multiple detection is in most cases inevitable: combinations of different concentration detectors provide information on copolymer composition, and molar mass-sensitive detectors can eliminate errors with inadequate calibrations. For complex polymers (with distributions in molar mass, chemical composition, functionality, etc.) one-dimensional techniques can, however, only provide part of the desired information. For these samples, multidimensional separations will be required. In most cases, one of the dimensions which will be SEC, while the other(s) could be gradient HPLC, or liquid chromatography at the critical point of adsorption (LCCC).

See also: **II/Chromatography: Liquid:** Derivatization; **Detectors:** Refractive Index Detectors; Evaporative Light Scattering Detectors in Liquid Chromatography. **Detectors:** Ultraviolet and Visible Detection; **III/Polymers:** Field Flow Fractionation.

Further Reading

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